PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 47/48

A2

(11) International Publication Number: WO 00/10610

(43) International Publication Date: 2 March 2000 (02.03.00)

(21) International Application Number: PCT/GB99/02606

(22) International Filing Date: 19 August 1999 (19.08.99)

(30) Priority Data:

9818027.6	19 August 1998 (19.08.98)	GB
9818156.3	20 August 1998 (20.08.98)	GB

(71) Applicant *(for all designated States except US)*: THERAMARK LIMITED [GB/GB]; 90 Fetter Lane, London EC4A 1JP (GB).

(71) Applicant *(for US only)*: ADAMS, Margaret (legal representative of the deceased inventor) [GB/GB]; 59 Ladder Hill, Wheatley, Oxford OX33 1HY (GB).

(72) Inventor: ADAMS, Ged (deceased).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BLAKE, David [GB/GB]; 5 Church Street, Brill, Aylesbury, Buckinghamshire HP18 9RT (GB). NAUGHTON, Declan [GB/GB]; Petite France Cottage, Petite France, Badminton, South Gloucestershire GL19 1AF (GB). STRATFORD, Ian [GB/GB]; Bretton Cottage, Bretton, Eyam, Hope Valley, Derbyshire S32 5QD (GB).

(74) Agent: ATKINSON, Peter, Birch; Marks & Clerk, Sussex House, 83–85 Mosley Street, Manchester M2 3LG (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: DRUG TARGETING

(57) Abstract

The use of a bioreductive conjugate comprised of a non–cytoxic bioreductive moiety having linked thereto at least one therapeutic agent, and salts thereof, is disclosed for the healing of wounds and the treatment of fibrotic disorders, ulcerative colitis, inflammatory bowel disease, epilepsy, cardiovascular reperfusion injury, cerebral reperfusion injury, hypertensions, cystic fibrosis, psoriasis, para–psoriasis, peptic ulcers, gastric ulcers, duodenal ulcers, diabetic ulcers dementia, oncology, Aids, rheumatoid arthritis, diabetes, and ischemia. Various specific conjugates for treating these conditions are also disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

DRUG TARGETING

The present invention relates to bioreductive drug conjugates for use in targeting of therapeutic agents to localised regions of hypoxic and/or ischemic tissue within the body.

Reduced oxygen tension (hypoxia) has been demonstrated in a variety of medical conditions. Thus for example, it has been demonstrated to be present in tumours and in fact it has long been suspected that oxygen deficiency in tumours may be a limiting factor in the control of tumours by radiotherapy. Furthermore, it is disclosed in WO-A-9912547 (Theramark Ltd) that in patients suffering from rheumatoid arthritis (a common systemic inflammatory disease which predominantly affects the synovial joints) the synovial tissues are profoundly hypoxia and contain high levels of reductases. Hypoxic tissue is also a feature of various fibrotic disorders and psoriasis.

Relatively recently, the presence of hypoxia in tumours has been exploited in their treatment by the use of bioreductive drugs, i.e. drugs which require metabolic reduction to generate cytotoxic metabolites. This process is facilitated by the presence of appropriate reductases and the lower oxygen conditions present in some cancerous (hypoxic) as compared to normal (normoxic) tissue. As a result, a number of bioreductive drugs capable of producing cytotoxic metabolites under hypoxic conditions have been proposed for use in combination with radiotherapy treatment of tumors.

A number of bioreductive compounds are known to act as potent alkylating agents after undergoing reduction *in vivo*. Examples of known bioreductive alkylating agents include compounds such as activated enamines, vinylogous quinone methides, simple quinone methides and α-methylene lactones or lactams. Bioactivation of such

compounds produces species which are electron deficient and which are capable of covalent binding to a nucleophilic centre on a biomolecule, such as DNA.

Most bioreductive drugs that have been developed for use in the treatment of tumors exhibit an optimum "trapping" potential when hypoxia is profound ($pO_2 < 12$ mm Hg) and this is believed to form the basis for their selectivity for cancerous as opposed to normal tissues.

Bioreductive drugs have also been proposed for use in several methods for the detection of hypoxic cells in tumors. In this way, radiotherapy treatment may be optimised for individual patients on the basis of the oxygen status of their tumors. Thus, for example, US-A-5086068 describes the use of nitroaromatic compounds in the detection of hypoxic cells in normal and tumor tissue. An immunogenic conjugate comprising a nitroaromatic compound and an immune response inducing carrier is used *in vitro* to raise antibodies specific to the nitroaromatic compound. These antibodies are in turn used to detect the presence of hypoxic tissue following *in vivo* administration of the nitroaromatic compound.

A number of methods have also been described for detecting the presence of hypoxic cells in tumors using a labelled 2-nitroimidazole in which labelled fragments of the nitroimidazole compound bind to cellular macromolecules. More recently, the use of an immunologically detectable hapten such as theophylline covalently bound to a 2-nitroimidazole has been suggested as a method of identifying hypoxic cells (see Brit. J. Cancer 63: 119-125, 1991 & 72: 1462-1468, 1995, and Anti-Cancer Drug Design 10: 227-241, 1995). Bioreduction of the nitroamidazole leads to binding of bioreductive metabolites, and hence the theophylline side-chain, to intracellular molecules. Immunochemical techniques are then used to stain and thus locate those cells containing the bound theophylline.

Other agents comprising a bioreductive moiety, e.g. 2-nitroimidazole, for the diagnosis or treatment of hypoxic cells are described in US-A-5387692.

A number of bioreductive agents have been described for use in the delivery of cytotoxic drugs to hypoxic tumor tissue in which bioreductive activation at the tumor site results in selective delivery of the drug. However, following drug delivery the bioreductive compound remaining in the tissues is itself a potential alkylating agent and thus cytotoxic, thereby rendering such a system entirely unsuitable for use as a non-cytotoxic drug delivery vehicle in diseases other than cancer. Hypoxia-selective bioreductive drug delivery agents proposed for use in anti -tumor therapy are described, for example, in Dissabs 87: 31004, 1987 and in J Med. Chem. 34: 2933-2935, 1991.

Delivery systems which utilise bioreduction to deliver a non-cytotoxic drug species have also been proposed. For example, a delivery system based on quinone propionic acid has been described (see Pharmaceutical Research 8(3): 323-330, 1991) in which the benzoquinone acts as the trigger and the propionic acid moiety allows for linkage either to an amine moiety (e.g. an enzyme inhibitor) or to an alcohol (e.g. a steroid). Two electron activation of the benzoquinone trigger facilitates intramolecular cyclisation generating a stable lactone, a process which results in elimination of the drug species. However, the lactone produced is itself a potential alkylating agent. This system is thus unsuitable for use as a non-cytotoxic drug delivery system. Furthermore, in aqueous solution in the absence of a reducing agent the lactone produced following drug delivery is very unstable and undergoes degradation. The instability of this prodrug system in aqueous solution thus precludes its use for drug delivery *in vivo*.

WO-A-98/35701 (PCT/GB98/00461 - Theramark Ltd.) discloses bioreductive conjugates comprising a non-cytotoxic bioreductive moiety with at least one therapeutic agent linked thereto which is intended to be released at a hypoxic site.

The conjugate is such that after release of the therapeutic agent the bioreductive moiety is itself a stable non-cytotoxic species or reacts with itself to form a stable, non-cytotoxic species. This minimises direct interaction of the carrier with DNA or other biomolecules thus avoiding potential mutagenic side effects. Thus, for example, in preferred conjugates as disclosed in the PCT application the bioreductive moiety is a benzoquinone nucleus. On reduction of the benzoquinone nucleus at the hypoxic site, the therapeutic agent is released and the residue of the bioreductive moiety participates in an intramolecular rearrangement and/or cyclisation reaction to generate a non-cytotoxic species. More specific examples of such conjugates are set our below.

The present invention relates to developments of the subject matter of the aforementioned PCT application.

1. First Aspect

In a first aspect, the present invention extends the range of conditions to which the drug conjugates of the aforementioned PCT application may be applied to include the healing of wounds, and the treatment of fibrotic disorders, ulcerative colitis, inflammatory bowel disease, epilepsy, cardiovascular reperfusion injury, cerebral reperfusion injury, hypertensions, cystic fibrosis, psoriasis, para-psoriasis, peptic ulcers, gastric ulcers, duodenal ulcers, diabetic ulcers dementia, oncology and AIDS.

Further exemplification of this aspect of the present invention in the above conditions and agents which may be used is given below.

1.1. Wound Healing and Regulating Fibrosis

It is often desirable to increase the rate of healing in the case of acute wounds (such as penetrative injuries, burns, nerve damage or even wounds resulting from elective surgery), chronic wounds (such as diabetic, venous and decubitus ulceration) or for generally healing compromised individuals (for example the elderly). In these

examples. the wounds can severely influence quality of life or even result in death and therefore the rate of healing often needs to be increased as much as is clinically possible. Where the rate of wound healing is increased, there is often an associated increase in scar formation but this may be of secondary importance compared to the desired increase in the rate of healing.

There are however other instances of wound healing in which fibrosis is regarded as a major problem in that the scar tissue which forms is not only unsightly but also causes problems in respect of growth, tissue functioning, movement etc. This is particularly true following injuries to children or following major burns. There are therefore situations where the regulation of scar formation is of primary importance and the rate of healing is only of secondary consideration. Examples of such situations are external wounds (especially of the skin) where excessive scarring may be detrimental to tissue function (for instance skin burns and wounds which impair flexibility of a joint). The reduction of scarring when cosmetic considerations are important (e.g. skin wounds of the face) is also highly desirable. In the skin, hypertrophic or keloid scars (particularly common in afro-Caribbean and mongoloid races) can cause functional and cosmetic impairment.

As well as external wounds (such as of the skin), internal scarring or fibrosis can be highly detrimental and specific examples include:

(i) Abdominal or peritoneal adhesions or strictures of the gut which may be life threatening scars or fibrotic conditions.

(ii) Scarring or fibrosis in the central nervous system (e.g. following a stroke or neurosurgery) which often leads to functional impairment and may inhibit neuronal reconnection.

(iii) Scarring or fibrosis in the eye (e.g. following injury or surgery of the cornea) may lead to visual impairment. For instance, scarring or fibrosis of the eye following glaucoma surgery can lead to a failure of the pressure equalising operation and may lead to a return of the disease conditions.

(iv) Fibrosis or scarring of ligaments or tendons can have serious effects on function.

Related to the above is the fact that there are a number of medical conditions in which excessive fibrosis leads to pathological derangement and malfunctioning of tissue. Examples include cirrhosis of the liver, glomerulonephritis, pulmonary fibrosis, scleroderma, systemic fibrosis, rheumatoid arthritis and proliferative vitreoretinopathy, in addition to wound healing. Systemic fibrosis may occur following wounding, ischaemia or some other pathological damage e.g. post-stroke scarring/ fibrosis in the central nervous system, cardiac scarring / fibrosis following myocardial infarction. The present invention which may be used for the treatment of such conditions by regulating (i.e. preventing, inhibiting or reversing) fibrosis or scarring.

Whilst the above considerations mainly apply to conditions of man it will be appreciated that wound healing, scarring and fibrosis can also be problematic in other animals (especially domestic animals such as horses, dogs, cats etc). For instance abdominal wounds or adhesions are a major reason for having to put down horses, as are tendon and ligament damage leading to scarring or fibrosis.

In a first embodiment of the invention, the therapeutic agent may be a growth factor neutralising agent or agents specific against only fibrotic growth factors. The growth factor neutralising agent may be a growth factor neutralising antibody, for example antibodies to TGF-ß1, TFG- ß2, PDGF, IFNγ or IL-1.

The growth factor neutralising agent may be a growth factor receptor blocking agent, for example a peptide containing the receptor binding site of the growth factors TGF-ß1, TFG- ß2, PDGF, IFNγ or IL-1

The growth factor neutralising agent may also comprise a molecule which binds to the growth factor to inhibit receptor binding. For example when the growth factor is selected from TGF-ß1, TFG-ß2, PDGF, IFNγ or IL-1, the molecule may be selected from Decorin, Biglycan, Fibromodulin, Lumican, Betaglycan, soluble type II TFG-ß Receptor and fragments or derivatives of these molecules which have binding affinity for the growth factors.

The growth factor neutralising agent may be an antisense oligonucleotide or ribozyme(s) to growth factor mRNA which both act to prevent mRNA from being translated.

The growth factor neutralising agent may also be a soluble form of the receptor or the growth factor binding domain of the receptor.

The growth factor neutralising agent may also be an aptmer which binds and neutralises the growth factor.

This embodiment of the invention is useful for inhibiting scar tissue formation during healing of wounds.

Examples of products which may be used in accordance with the first embodiment of the invention are disclosed in WO-A-92/17206, the disclosure of which is incorporated by reference.

In a second embodiment of the invention, the therapeutic agent is a non-fibrotic growth factor which may, for example, be TGFß-3, FGF-1, FGF-2, IL-4 or IL-10. Such products are useful particularly for preventing, inhibiting or reversing fibrosis. If desired, the gene product used in the second embodiment of the invention may be co-expressed with at least one anti-fibrotic agent, for example anti-TGFß-1/TGFß-2.

This embodiment of the invention is useful for inhibiting fibrosis during the healing of wounds and in other fibrotic conditions and disorders.

Further details as to gene products which may be used in accordance with the second embodiment of the invention are disclosed in WO-A-93/19769, the disclosure of which is incorporated by reference.

In accordance with a third embodiment of the invention the therapeutic agent is one which is capable of affecting the quantity of active growth factor or a protein associated therewith in a wound site at which the gene product is expressed. The agent may, for example, be specific to a non-fibrotic growth factor, e.g. selected from FGF-1, FGF-2, FGF-7, EGF, TGFα, IL-4, IL-10, IL-12, IL-17 or TGF-β_3. Alternatively, the agent may be specific to a fibrotic growth factor, e.g. TGF-β_1, TGF-β_2, PDGFAA, PDGFBB, PDGFA, a member of the CTGF family, IL-1, IL-2, IL-6, IL-8 and TFNα.

This embodiment of the invention may be used to promote the healing of wounds or fibrotic disorders with reduced scarring.

Further details relating to the third embodiment of the invention are given in WO-A-95 26203, the disclosure of which is incorporated herein by reference.

In a fourth embodiment of the invention, the therapeutic agent may be IL-4 or IL-10 or a fragment or a partially modified form thereof. By "fragment or partially modified form thereof" is meant a fragment or partial modified form of IL-4 or IL-10 which retains the anti-inflammatory healing functionality of IL-4 or IL-10.

IL-4 and IL-10 as well as fragments and partially modified forms thereof promote the healing of wounds or fibrotic disorders with reduced scarring as disclosed

more fully in WO-A-97/05894 (PCT/GB96/01930), the disclosure of which is incorporated herein by reference.

In a fifth embodiment of the invention the therapeutic agent is a soluble betaglycan or a fragment or an analogue thereof which may be used for the healing of wounds or fibrotic disorders with reduced scarring. By "fragment or analogue" is meant a molecule which is capable of binding to TGF-β_2 performing the same role as soluble betaglycan. The "fragment or analogue" may, for example, comprise at least the TGF-β binding fragment of soluble betaglycan.

This embodiment of the invention is useful for the treatment of wounds or fibrotic disorders with reduced scarring.

Reference is made to WO-A-97/05883 (PCT/GB 96/01840) for further disclosure relating to the use of soluble betaglycan or fragments or analogues thereof, the disclosure of GB 9516073.5 being incorporated herein by reference.

In sixth embodiment of the invention, the therapeutic agent is an inhibitor of Interferon-γ (IFN-γ).

The inhibitor may, for example, be a neutralising antibody. Alternatively, the inhibitor may be anything which inhibits IFN-γ from interacting with its receptor. It may, for example, be a molecule which mimics the IFN-γ receptor binding sequence and which binds to the receptor but does not activate it, thereby competitively inhibiting the binding of IFN-γ to the receptor and inhibiting the activation of the receptor.

This embodiment of the invention is useful for promoting the healing of wounds or fibrotic disorders with reduced scarring.

In an seventh embodiment of the invention. the therapeutic agent may be a stimulator of IFN-γ, i.e. an agent which increases the quantity or the efficacy of active IFN-γ at a site. This may be IFN-γ itself or an analogue of IFN-γ. Alternatively, it may be an inhibitor of IFN-γ metabolism.

This embodiment of the invention is useful for promoting the healing of chronic wounds.

Further details relating to the sixth and seventh embodiments of the invention are disclosed in WO-A-97/07136, the disclosure of which is incorporated herein by reference.

In a eighth embodiment of the invention the therapeutic agent is an inhibitor of activation of at least one integrin receptor.

The inhibitor may bind to at least one receptor but not activate it.

The inhibitor may comprise an antibody. It may comprise an neutralising antibody. The antibody may bind specifically to at least one integrin receptor. It may bind specifically to the RGD peptide or an analogue thereof.

The inhibitor may comprise at least the RGD peptide or an analogue thereof.

The inhibitor may be any form of inhibitor which inhibits the activation of at least one integrin receptor. It may, for example, be a neutralising antibody specific to the RGD peptide of integrins, it may be a neutralising antibody specific to the integrin receptor, or it may contain the RGD peptide or an analogue (e.g. a RGDS peptide or a mimitope of RGD) thereof which will bind to the integrin receptor and prevent the natural ligand from binding to it.

The receptor may be the GpIIb/IIIa platelet receptor. Therefore the inhibitor may be a GpIIb/IIIa platelet receptor inhibitor. The inhibitor may also comprise an RGD peptide or an analogue thereof.

The inhibitor may inhibit the binding of TGF-β_1 and/or platelets or leukocytes to fibrin and/or fibrinogen and/or fibronectin. It may for example be a fibrinogen receptor antagonist.

This embodiment of the invention is useful for the healing of wounds or fibrotic disorders with reduced scarring.

Further details relating to the eighth embodiment of the invention is given in WO-A-97/11718 (PCT/GB 96/02366), the disclosure of which is incorporated herein by reference.

In accordance with a ninth embodiment of the present invention, the therapeutic agent is an inhibitor of at least one convertase enzyme.

The inhibitor of the convertase enzyme may be a serine protease inhibitor.

This embodiment of the invention is useful for promoting the healing of wounds or fibrotic disorders with reduced scarring.

In accordance with a tenth embodiment of the present invention, the therapeutic agent may be a stimulator of Activin and/or Inhibin.

By "stimulator" is meant anything which may stimulate the quantity or efficacy of active activin and/or active inhibin at a site. This may be activin or inhibin itself or an analogue thereof. Such an analogue may, for example, have a longer half-life than activin or inhibin, or it may have a different binding affinity for its receptors.

A fragment may comprise at least that part of activin or inhibin which is required to allow it to stimulate its receptors. Alternatively, it may, for example, be an inhibitor of activin metabolism or it may be a stimulator of activin synthesis. For example, it may be analogue of a fragment of activin or inhibin which is bound by a degraditive enzyme. It may be a mimotope made to a fragment of activin or inhibin which is bound by an enzyme which degrades it. Such a mimotope combined to the receptor site of the enzyme, competitively inhibiting the binding of activin or inhibin (as appropriate) to the enzyme and thereby inhibiting is degradation.

The stimulator may be an antagonist of an agonist of Activin and/or Inhibin. For example, the stimulator may be an antagonist of Follistatin.

This embodiment of the invention is useful for promoting the healing of wounds and fibrotic disorders with reduced scarring.

Further details regarding the tenth embodiment of the invention are given in WO-A-97/15321 (PCT/GB 96/02559), the disclosure of which is incorporated herein by reference.

In accordance with a eleventh embodiment of the present invention the therapeutic agent is one which modulates actin assembly and organisation. The product may for example be Gelsolin, Villin, CaPG, adseverin, flightless-1, advillin or derivatives thereof.

This embodiment of the invention is useful for increasing the rate of wound healing as well as improving scar quality.

Further details regarding the eleventh embodiment of the invention are disclosed in WO-A-98/24465, the disclosure of which is incorporated herein by reference.

In accordance with an twelfth embodiment of the present invention the therapeutic agent may be an agent which inhibits the activity of Interleukin-6.

Suitable inhibitors of IL-6 activity and thereby preferred proteins for use according to the twelfth embodiment of the invention include IL-6 Receptor antagonists (compounds which inhibit receptor activation by IL-6); compounds that disrupt signalling mediated by IL-6 (e.g. inhibitors of second messenger production, kinase inhibitors or modulators of gene expression); enzymes that specifically degrade IL-6 and inhibitors of IL-6 synthesis, neutralising antibodies to IL-6 (which would normally be high affinity antibodies used at a high concentration because low affinity/low concentrations of neutralising antibody are known to act as carriers and protective agents and so potentiate the activity of IL-6 (Heremans et al. Eur. J. Immunol. 22 p2395-2401, 1992), antisense oligonucleotides or ribozymes to IL-6, oligonucleotide aptmers which bind to and neutralise IL-6 or its receptor, molecules which bind to IL-6 and increase its clearance from a wound site.

The most preferred compounds for use as gene products for use according to the fifteenth embodiment of the invention are IL-6 Receptor antagonists and disrupters of IL-6 signalling.

This embodiment of the invention is useful for reducing fibrosis in wound healing and treatment of fibrotic disorders.

Further details relating to the twelfth embodiment of the invention are given in WO-A-98/36061 (PCT/GB98/00319), the disclosure of which is incorporated herein by reference.

In accordance with a thirteenth embodiment of the invention the therapeutic agent is Latency Associated Peptide or a functional analogue thereof.

This embodiment of the invention is useful for promoting wound healing.

Further details relating to the thirteenth embodiment of the invention are given in WO-A-98/35695 (PCT/GB98/00316), the disclosure of which is incorporated by reference.

In accordance with a fourteenth embodiment of the invention the therapeutic agent is Insulin Like Growth Factor II or a functional analogue thereof.

This embodiment of the invention is useful for promoting the rate of wound healing and for reducing or preventing scar formation and fibrosis.

In accordance with a fifteenth embodiment of the invention the therapeutic agent is a compound that influences the sex hormone system. The agent may be one which promotes oestrogen activity at the site of a wound for accelerating the healing of the wound. The agent promoting oestrogen activity may for example be oestrogen. Alternatively, the therapeutic agent may be one which modulates and androgenic activity, e.g. by promoting androgenic activity for accelerating the healing of wounds or by inhibiting androgenic activity for inhibiting fibrosis. Alternatively the therapeutic agent may be one which promotes progesterone activity for inhibiting fibrosis.

Further details relating to the fifteenth embodiment of the invention are given in WO-A-98/03180, the disclosure of which is incorporated herein by reference.

1.2 Ulcerative Colitis and Inflammatory Bowel Disease

The therapeutic agent which may be used in this embodiment of the invention include Sulphasalazine (and other 5-aminosalicylates), Metronidazole,

Corticosteroids. Azathioprine, Cyclosporin A, and Methatrexate. Other agents would include ulcer healing drugs such as Omeprazole, Lansoprazole, and Rabeprazole.

1.3. Epilepsy

The therapeutic agent for use in the treatment of epilepsy may for example be Phenytoin, Phenobarbitone, Sodium Valporate, Topiramite.

1.4. Reperfusion Injury and Hypertension

Therapeutic agents which may be used in the treatment of these conditions include

1. Phosphodiasterase inhibitors
2. Modulators of immune response/apoptosis
3. Vasodilators, such as
   a) nitrates e.g. Isosorbide mono- or di-nitrate, or Glyceryl tri-nitrate, or,
   b) calcium antagonists, such as Verapamil/nifedipine, and Diltiazem
4. ACE inhibitors, such as Trandolapri, Captopril
5. Fibrinolytic agents, such as Streptokinase, Activase.
6. Anti platelets, such Aspirin, Ticolpidine.
7. Anti coagulants, such as Wolferin.
8. Beta blockers, such as Atenolol, Propranolol.
9. Xanthene Oxidase inhibitors, such as Elopurinol.
10. Free radical scavangers, such as Vitamin E, Manetol.

1.5. Cystic Fibrosis

Therapeutic agents which may be used in the treatment of these conditions include Ibuprofen and Prednisolone.

1.6. Psoriasis

Therapeutic agents which may be used in the treatment of these conditions include

1. Steroids such as Hydrocortisone, Prednisolone.
2. Vitamin D analogues
3. Psoralens
4. Antimitotics/immunosuppressants, such as Methotrexate, Retinoids, Cyclosporin A.

2. Second Aspect

In a second aspect, the present invention extends the range of therapeutic agents (in the drug conjugates) which may be used in the treatment of conditions disclosed in WO-A-98/35701, namely rheumatoid arthritis, diabetes, Crohn's Disease and ischaemia.

2.1. Rheumatoid Arthritis

Therapeutic agents which may be used in the treatment of these conditions include Sulfasalazine, Mesalazine, Penicillamine, Azathioprine, Chlorambucil, Myochrysine (sodium auro thiomalate), Hydroxychloroquine, Methotrexate, Cyclosporin Myocrisin and Neoral.

2.2. Diabetes

Therapeutic agents which may be used in the treatment of this condition include Acarbose, Aspirin, Indomethacin, Capropril and Prostaglandin Synthetase inhibitors.

2.3. Ischemia

Therapeutic agents that may be used in the treatment of this condition include peripheral vasodilators, such as Inositol Nicotinate, calcium antagonists, such as Niphedipine and Verapamil; anti platelets, such as Aspirin and Dipyridamole, ACE

inhibitors. (Agniotensin Converting Enzyme) e.g. Ramapril and Trandolapril, fibrinolotic agents.

3. Third Aspect

In a third aspect, the present invention extends the range of non-steroidal anti-inflammatory agents and PDE inhibitors which are disclosed as class of drugs in the WO-A-98/35701.

3.1. Non-steroidal anti-inflammatory agents

Examples of these agents which may be used include Ibuprofen, Naproxen, Fenoprofenb. Benoxaprofen, Sulindac, indomethacin, tolmetin and Diclofenac.

3.2. PDE Inhibitors

PDE

These may include, for example, PDE-4 inhibitors (Rolipram) or DEC-5 inhibitors. such as Zapronist, Dipyridamole or Sildenafil.

4. Fourth Aspect

In accordance with a fourth aspect of the present invention, the conjugates of WO-A-98/35701 in which the therapeutic agent is a PDE inhibitor (preferably a PDE-5 inhibitor) are used for the treatment of hypoxic conditions such as diabetes, rheumatoid arthritis, cancer and other hypoxic conditions as disclosed in the present specification or WO-A-98/35701.

5. Fifth Aspect

In accordance with a fifth aspect of the present invention there are provided bioreductive conjugates comprising a bioreductive moiety of the type disclosed in WO-A-98/35701 in conjunction with a therapeutic agent which is selected from immunosupressives, cell cycle specific drugs, cell cycle non-specific drugs, metalloprotease inhibitors and inhibitors of nitric oxide synthase.

The conjugates of this fifth aspect may be used as appropriate for any therapeutic application as disclosed in WO-A-98/35701, the present application, or as appropriate for any other therapeutic application.

In the case where the therapeutic agent is an immunosupressive, the bioreductive conjugate may be used in transplant surgery. The immunosupressive may, for example, be cyclosporin A.

Examples of cell cycle specific and cell cycle non-specific drugs include hormones and hormone analogues, anti-angiongenic, (e.g. endostatin, angiostatin), vascular targeted drugs (e.g. combreastatin), metalloprotease inhibitors.

The treatment of periodontitis may be effected using a bioreductive conjugate for which the therapeutic agent is a metalloprotease inhibitor.

The treatment of sepsis may be effected with an inhibitor of nitric oxide synthase.

The bioreductive conjugates used in accordance with the invention are substantially stable in an oxygenated environment. However in a hypoxic or ischemic environment, reductive activation results in release of the therapeutic agent from the bioreductive moiety and thus its targeted delivery to the site of hypoxia or ischemia which may be an organ, tissue, cell or group of cells. In general, on bioreduction the bioreductive intramolecular cyclisation reaction which in turn provides for release of the therapeutic agent at the target site.

As used herein, the term "bioreductive moiety" is intended to define any molecule which is reduced in the presence of reducing enzymes or reductases. For example, a bioreductive moiety may be any substantially non-reactive molecule which in the presence of reductases is converted into a more reactive for. Preferred

bioreductive moieties for use in the invention are those which on reductive activation become electron-rich and which are thereby capable of intramolecular bon rearrangement to deliver a therapeutic agent.

As used herein, "non-cytotoxic bioreductive moiety" is used to define any bioreductive moiety having substantially no cytoxic activity *in vivo*. Thus, it is intended that the bioreductive moiety for use in accordance with the invention is not only in itself non-cytoxic. but that this produces substantially no cytoxic species following bioreductive activation. By "non-cytoxic" it is really meant that the bioreductive moiety does not interact directly with DNA. Preferably, the bioreductive moiety is substantially non-mutagenic. Thus, the bioreductive moiety is intended to function merely as a non-cytoxic carrier or targeting agent for the drug species which, following delivery of the drug at the target site, is eliminated from the body in the absence of any undesirable side-effects.

The bioreductive conjugates in accordance with the invention having a targeting effect on tissues having reductase activity. This is believed to be a consequence of hypoxic metabolism and/or reduced oxygenation of such tissues.

The bioreductive conjugate used in accordance with the invention may be of formula (I):

$$A(B)_n \qquad (I)$$

where A is a non-cytoxic bioreductive moiety, each B is independently the residue of a therapeutic agent, and n is an integer, preferably from 1 to 3, particularly 1.

A and B are stably conjugated in an oxygenated environment and are such that A is non-cytoxic and B when conjugated to A is non-cytoxic. On reductive activation

of A, A and B detach and A is itself either a stable, non-cytoxic species or, more preferably, A reacts with itself to form a stable, non-cytoxic species.

Preferred compounds for use in accordance with the invention are those which have the ability to penetrate poorly perfused tissues and which only release the active drug in a hypoxic and/or ischemic environment.

A large number of bioreductive agents of diverse structure are known. These include quinones, aromatic nitro compounds and N-oxides. As mentioned above, those intended for use in accordance with the invention should be substantially non-cytotoxic following bioreductive activation. This may be achieved in a number of ways.

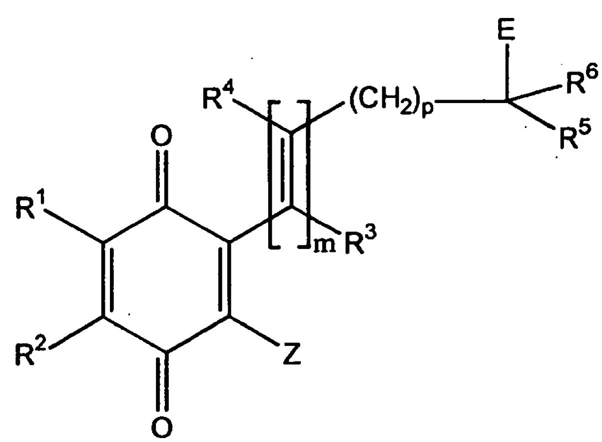
Following bioreduction of the conjugate and delivery of the drug species to the target site, the final form of the bioreductive carrier may itself comprise a stable, non-cytotoxic species, for example a compound having no potential alkylating centre. However, in a preferred embodiment of the invention, cytotoxicity of the bioreductive moiety may be reduced by providing a nucleophilic centre within the bioreductive compound itself. Following release of the drug an alkylating centre is formed. However, the proximity of the nucleophilic centre ensures that intramolecular alkylation occurs in preference to alkylation of any biomolecules such as DNA. In this way, substantially no cytotoxic species are formed. Such systems may be referred to as "self–alkylating".

Examples of electron rich groups capable of acting as a nucleophilic moiety in the bioreductive compound include oxygen, sulphur and nitrogen atoms. Thus, for example, inclusion of a suitably positioned amino, thio or hydroxyl group within the bioreductive compound will favour intramolecular alkylation resulting in a non-cytotoxic product on release of the drug at the site of hypoxia/ischemia. Suitable nucleophilic moieties which may be present in the bioreductive moiety include -OH,

-SH, $-NH_2$ and -NHR in which R is C_{1-6} alkyl, e.g. C_{1-3} alkyl. Other suitable nucleophilic moieties will be known to those skilled in the art.

Alternatively, the bioreductive compound for use in the invention may be rendered non-cytotoxic following drug delivery by means of the introduction of steric hindrance capable of presenting a physical blockage to attack upon the bioreductive by any nucleophile. Thus, the presence of a bulky group either at or in close proximity to any potential alkylating centre generated in the bioreductive moiety following drug delivery serves to abolish alkylating reactivity thus preventing alkylation of any biomolecules. Examples of groups which may be used in this way include linear or, more preferably, branched, C_{4-20} alkyl or alkenyl groups, e.g. tert. butyl. Other groups capable of providing steric hindrance will be known to those skilled in the art.

Particularly preferred bioreductive conjugates in accordance with the invention include compounds of formula II:



(II)

(wherein

R^1 and R^2 independently represent hydrogen or halogen atoms, or a group R, OR, SR, NHR, NR_2, CO_2R or CONHR;

or, alternatively, R^1 and R^2 together with the intervening ring carbon atoms form a 5-7 membered, preferably 5- or 6-membered, carbocyclic or heterocyclic ring itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, $C0_2R$ and CONHR;

Z represents an alkyl, alkenyl, aryl or aralkyl group optionally carrying at least one OH, SH, NH_2 or NHR^7 group in which R^7 is an alkyl group;

R^3, R^4, R^5 and R^6 independently represent hydrogen atoms

or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L;

m = 0, 1, 2 or 3, preferably 1;

p = 0 or 2, preferably 0;

with the proviso that when m = 1 then p = 0)

or a salt thereof.

Preferred compounds of formula II include those wherein Z represents a group of the formula $(CH_2)_nXH$ in which n = 0, 1, 2 or 3, preferably 0; and X represents an oxygen or sulphur atom or, preferably, X represents a group of formula NY wherein Y

represents a hydrogen atom or an alkyl group. Such compounds may act as "self-alkylating" systems.

Particularly preferred compounds of formula II are those wherein Z represents a group of the formula $(CH_2)_nXH$ in which X represents an amino group;

R^1 and R^2 each represent alkoxy groups or, together with the intervening ring carbon atoms, R^1 and R^2 form a benzene ring;

R^3, R^4, R^5 and R^6 each represent hydrogen atoms; and

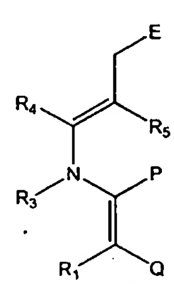
$n = 0$, $m = 1$ and $p = 0$.

Alternatively, in relation to the compounds of Formula II, particularly when Z is other than a group of the formula $(CH_2)_nXH$, reduction of the quinone to its hydroquinone for may facilitate an intramolecular cyclisation reaction via the hydroxy group present on the hydroquinone ring and subsequent elimination of the drug species. The resulting cyclic ether is non-cytotoxic.

Reaction scheme 1 below illustrates the preparation of a preferred bioreductive conjugate for formula II in which R^1, R^2 and Z are as hereinbefore defined. As will be seen, bioreductive activation of the conjugate results in the formation of a cyclic ether which is an analogue of vitamin E and non-cytoxic.

Other preferred bioreductive conjugates in accordance with the invention include those compounds of formula III:

(III)



(wherein

P and Q together with the intervening ring carbon atoms form a quinone or indoloquinone ring, a nitroaromatic, N-oxide or diazoaromatic compound, itself optionally substituted by one or more halogen atoms, or one by one or more groups selected from R, OR, SR, NHR, NR_2, CO_2R and CONHR;

R represents a hydrogen or halogen atom, or a group R, OR, SR, NHR, NR_2, $C0_2R$ or CONHR;

R^3, R^4 and R^5 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L);

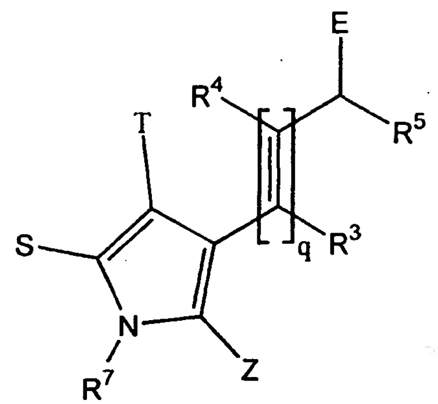
or a salt thereof.

Preferred compounds of formula III are those wherein P and Q together with the intervening ring carbon atoms form a quinone or indoloquinone ring; and

R^1, R^3, R^4 and R^5 each represent hydrogen atoms or methyl groups.

To act as "self-alkylating" systems, the electron-rich heteroatom present in the reduced form of the ring system of the compounds of formula III should preferably be no more than 6 bonds from the carbon atom linked to the therapeutic agent, E.

Other preferred bioreductive conjugates in accordance with the invention include the compounds of formula IV:



(IV)

(wherein

S and T together with the intervening ring carbon atoms form a quinone or iminoquinone ring, a nitroaromatic or N-oxide, e.g. an aromatic N-oxide, compound, itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, $C0_2R$ and CONHR;

Z represents an alkyl, alkenyl, aryl or aralkyl group optionally carrying at least one OH, SH, NH_2 or NHR^6 group in which R^6 is an alkyl group;

R^7 represents an alkyl group, preferably C1-2 alkyl;

R^3, R^4 and R^5 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

q = 0, 1, 2 or 3, preferably 0 or 1;

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L);

or a salt thereof.

Preferred compounds of formula IV are those in which S and T together with the intervening ring carbon atoms form a quinone or N-oxide compound:

R^3, R^4 and R^5 each represent hydrogen atoms;

R^7 is methyl;

Z represents a group of formula $(CH_2)_nXH$ wherein X

represents an oxygen or sulphur atom or, preferably, a group of formula NY in which Y represents a hydrogen atom or an alkyl group, and n = 0, 1, 2 or 3; and

q = 0 or 1.

In relation to the compounds of formula IV, alkylating activity may effectively be abolished following drug delivery by choosing as group Z a bulky group capable of providing steric hindrance. In such cases, Z is preferably a linear or, more preferably, branched, C_{4-20} alkyl or alkenyl group. Alternatively, such compounds may act as "self–alkylating" systems in cases where Z represents a group of the formula $(CH_2)_nXH$.

In each of the compounds of general formulae II-IV above, the substituents R, R^1, R^2 , R^3, R^4, R^5, R^6 and R^7 may be selected to provide the conjugate with optimum redox potential, solubility, enzyme specificity etc.

As used herein, the term "heterocyclic group" is intended to define a carbocyclic group interrupted by at least one heteroatom selected from oxygen, sulphur and nitrogen.

Examples of preferred carbocyclic or heterocyclic rings include benzene, pyridine, pyrrole, furan, pyrazine, piperidine, piperazine, pyrrolidine, morpholine and thiomorpholine rings.

In each of the compounds of formulae II-IV, preferred halogen atoms are fluorine and chlorine.

In the bioreductive conjugates of the invention, any alkyl or alkenyl moiety, unless otherwise stated, may be straight-chained or branched and preferably contains from 1 to 8, more preferably 1 to 6, and especially preferably 1 to 4, carbon atoms. Aryl moieties, unless otherwise stated, preferably contain from 5 to 12 ring atoms and especially preferably comprise phenyl rings.

Preferred salts of the compounds of formulae I-IV are those which are suitable for administration to patients and are thus pharmaceutically or physiologically acceptable salts. Such salts may be formed with various inorganic and organic acids and include the ammonium, alkali and alkaline earth metal salts.

Reductases known to be involved in activation of bioreductive compounds include DT diaphorase, cytochrome P450, NADPH-dependent cytochrome P450 reductase and xanthine oxidase. The ease of reduction of any given bioreductive agent will depend upon its ability to act as a substrate for the intracellular reductases and the expression levels of such enzymes within the particular cell type. The choice of bioreductive compound for use in the invention will thus depend upon the type of enzymes present at the target site. Indeed, it may be useful to determine the relative enzyme activities in the target tissues of individual patients before starting treatment.

It is clearly desirable that the bioreductive conjugate should reach the target site intact. Since bioreduction of the conjugate is dependent upon the redox potential of the bioreductive moiety present, this may be selected such that this is less susceptible to reduction by ubiquitous systems such as NADH or NADPH, thereby increasing the chances that the conjugate will reach the target site still intact. In general, those bioreductive compounds having an optimal redox potential will be more selective in targeting of hypoxic cells and are thus preferred for use in the invention.

Examples of bioreductive compounds preferred for use in the invention include the quinones, naphthoquinones, indoloquinones and quinolino quinones and their derivatives. The electron deficient quinone nucleus in such compounds readily

undergoes reduction *in vivo* to form the corresponding electron rich hydroquinone which in turn is capable of intramolecular rearrangement to release the drug. Particularly preferred quinones include the 1,4-benzoquinones and the naphthoquinones in which the quinone ring carries an optionally hydroxy or amino substituted alkenyl group e.g. a propenyl group, and an adjacent nucleophilic moiety, e.g. an amino group. Idoloquinones are particularly good substrates for DT diaphorase, an enzyme commonly found in most tissues.

The invention is considered to have utility in connection with the delivery if a wide range of therapeutic agents. The expression therapeutic agent" and "drug" are used interchangeably herein and are intended to define any atom. in or molecule which *in vivo* is capable of producing an effect detectable by any chemical, physical or biological examination. A therapeutic agent will in general be any substance which may be administered to a human or non-human animal body to produce a desired, usually beneficial, effect and may be an agent having either a therapeutic or a prophylactic effect.

Whilst it is envisaged that in general the therapeutic agent will itself be non-cytoxic, the bioreductive carrier may be used to deliver cytoxic agents, e.g. in anti-tumor treatment.

Methods for attaching bioreductive compounds to a therapeutic agent are within the level of skill in the art. In general, the conjugates in accordance with the invention can be prepared by linkage of a non-cytoxic bioreductive moiety to at least one therapeutic agent. Linkage of the therapeutic agent to the bioreductive moiety may be effected through any reactive group and standard coupling techniques are known in the art. Preferred reaction conditions, e.g. temperature, solvents, etc. depend primarily on the particular reactants and can readily be determined by those skilled in the art. In general, any reactive groups present, e.g. amino, carboxy etc. will be protected during coupling of the bioreductive with the therapeutic agent, although it is

possible to leave some groups unprotected. After coupling, the resulting compound may be purified. e.g. by chromatography.

The bioreductive moiety may be bonded directly to the therapeutic agent or may be bonded by a linker group, L. Linkage between the bioreductive and the therapeutic agent may be effected via any reactive group present in the bioreductive moiety, e.g. a primary

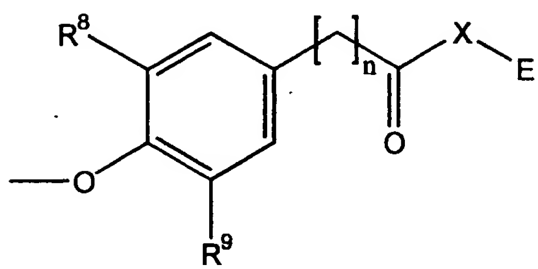
amine. carboxylate, alcohol, thiolate, etc. Preferably, the bioreductive moiety is linked to the therapeutic agent via an ester, phosphate ester, ether, amine, thiol or thiol ester bond or any combination thereof.

The linker group serves to link the bioreductive moiety to at least one therapeutic agent. Besides filling this role as a linker, the linker group may be selected to yield a bioreductive conjugate having desired characteristics. For example, appropriate choice of a linker group may serve to enhance the resistance of the conjugate to non-bioreductive metabolism and/or enhance delivery of the drug molecule at the target site. It may also be possible to optimise the redox potential, enzyme or tissue specificity, or the solubility of the conjugate by attaching to or incorporating within the linker group appropriately selected moieties, e.g. groups which are tissue targeting. Thus, the ability to alter the nature of the linker group provides for the possibility of altering the physicochemical properties, e.g. solubility, and biological properties, e.g. biodistribution, of the bioreductive conjugate. The primary function of the linker is however to link together the bioreductive compound and the drug.

Linker groups L particularly suitable for use in the invention for those drugs having a free -OH or -SH group include the following in which E represents the residue of a drug species:

-O-CO-$(CH_2)_n$-CO-X-E

and



(wherein n is an integer from 1 to 3;

X represent a sulphur or oxygen atom which may form part of the drug molecule E;

and R^8 and R^9 each independently represent F or Cl)

The bioreductive itself may be synthesised in accordance with conventional synthesis techniques. Techniques for the synthesis of quinones, in particular indoloquinones are described for example in J. Org. Chem. 50:4276-4281 (1985).

Pharmaceutical compositions for used in the invention comprise the bioreductive conjugate or a pharmaceutically acceptable salt thereof, together with at least one pharmaceutical carrier or excipient.

The active ingredient in such compositions may comprise from about 0.1% to about 99% by weight of the formulation. By "pharmaceutically acceptable" is meant that the ingredient must be compatible with other ingredients of the compositions as well as physiologically acceptable to the patient.

Pharmaceutical compositions for use according to the present invention may be formulated in conventional manner using readily available pharmaceutical or veterinary aids. Thus the active ingredient may be incorporated, optionally together with other active substances, with one or more conventional carriers, diluents and/or excipients, to produce conventional galenic preparations such as tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, soft and hard gelatin capsules, suppositories, sterile injectable solutions, sterile packaged powders, and the like.

Examples of suitable carriers, excipients, and diluents are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate

microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, water, water/ethanol, water/gylcol, water/polyethylene, glycol, propylene glycol, methyl cellulose, methylhydroxybenzoates, propyl hydroxybenzoates, talc, magnesium stearate, mineral oil or fatty substances such as hard fat or suitable mixtures thereof. The compositions may additionally include lubricating agents, wetting agents, emulsifying agents, suspending agents, preserving agents, sweetening agents, flavouring agents, and the like. The formulations may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by use of procedures well known in the art.

The compositions are preferably formulated in a unit dosage form, e.g., with each dosage containing from about 0.1 to about 500mg of the active ingredient.

The precise dosage of the active ingredient and the length of the treatment will depend upon a number of factors including the age and weight of the patient, the specific condition being treated and its severity, and the route of administration. In general, an effective dose will be of the order of from about 0.01 mg/kg to about 20 mg/kg bodyweight per day, e.g. from about 0.05 to about 10 mg/kg per day, administered one or more times daily. Thus, an appropriate dose for an adult may be from 10 to 100 mg per day, e.g. 20 to 50 mg per day.

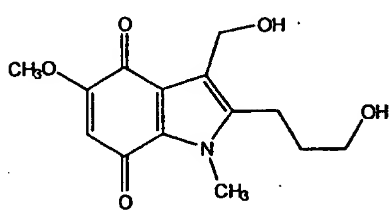
Administration may be by any suitable method known in the art, including for example oral, parenteral (e.g. intramuscular, subcutaneous, intraperitoneal or intravenous), rectal or topical administration.

EXAMPLE

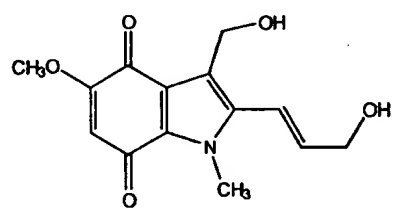
To illustrate the benefits of the use of bioreductive moieties in accordance with the invention, particularly with regard to reduced cytotoxicity, the following test was carried out on a number of model compounds.

A549 lung cancer cells were exposed to the model compounds for three hours in both aerobic and hypoxic conditions. After drug removal, the surviving cells were allowed to grow for 4 days before the number was determined using the MTT assay (I.G. Stratford and M.A. Stephens, International Journal of Radiation Oncology, Biology and Physics, Vol. 16. Pp. 973-976)

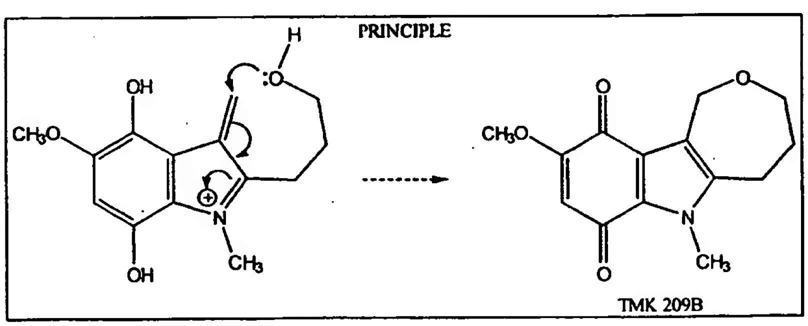
In a first series of tests, the following two compounds were evaluated:



TMK 209



E07 (known)

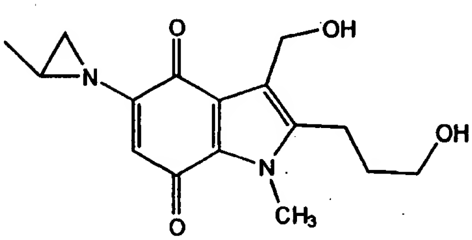


As indicated by the scheme shown in the box, compound TMK 209 is capable of undergoing self-alkylation whereas it would be appreciated that EO7 is not.

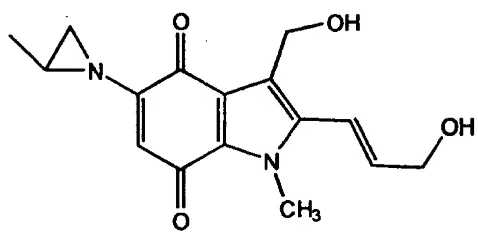
The following results were obtained:

	IC50	(μM)
Compound	**Air**	**N_2**
TMK 209	116	37
E07	45	2.8
	2.6	**13.2**

In a second series of tests, the following model compounds were used:

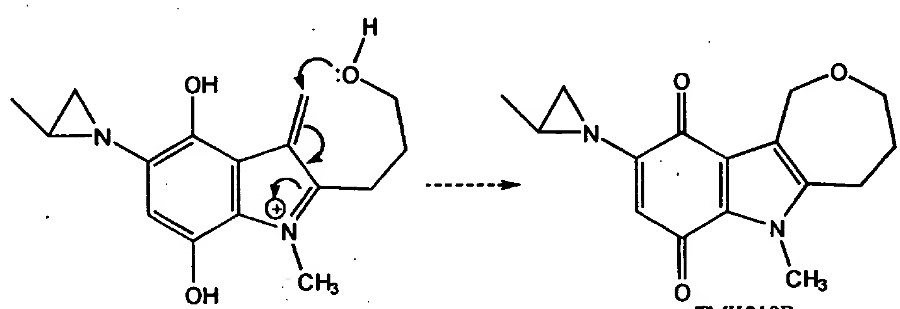


TMK210



E08

PRINCIPLE



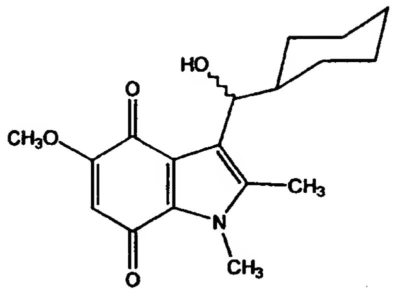
TMK210B

As we appreciated from the scheme shown in the box, compound TMK 210 is capable of undergoing self-alkylation but E08 is not.

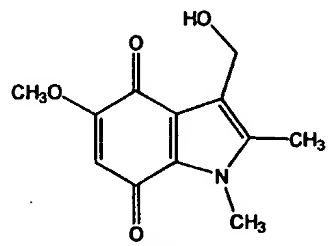
The results obtained were as follows:

	IC50	**(μM)**
Compound	**Air**	**N_2**
TMK 210	5.7	0.095
E08	1.4	0.016
	4.1	**5.9**

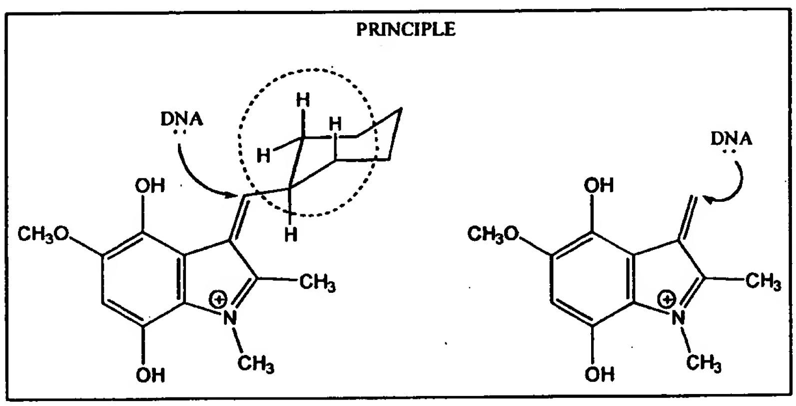
In a third series of tests, the following model compounds were used:



TMK 207



RB94547J (known)



As will be appreciated from the scheme shown in the box, compound TMK 207 is sterically hindered to prevent alkylation of DNA whereas RB94547J is not.

The following results were obtained:

	IC50	**(μM)**
Compound	**Air**	**N_2**
TMK 207	195	270
RB 94547	210	61
	0.93	**4.4**

The above results demonstrate the benefits of reduced cytotoxicity obtained using (as the residue of the bioreductive moiety) an entity which is either self-alkylating or which is sterically hindered.

CLAIMS

1. The use of a bioreductive conjugate comprising a non-cytoxic bioreductive moiety with linked thereto at least one therapeutic agent, and salts thereof, for use in the manufacture of a medicament for use in the healing of wounds, and the treatment of fibrotic disorders, ulcerative colitis, inflammatory bowel disease, epilepsy, cardiovascular reperfusion injury, cerebral reperfusion injury, hypertensions, cystic fibrosis, psoriasis, para-psoriasis, peptic ulcers, gastric ulcers, duodenal ulcers, diabetic ulcers dementia, oncology and AIDS.

2. The use as claimed in claim 1 wherein the medicament is for use in the healing of wounds or the treatment of fibrotic disorders.

3. The use as claimed in claim 1 wherein the therapeutic agent is a growth factor neutralising agent or agents specific against only fibrotic growth factors.

4. The use as claimed in claim 3 wherein the fibrotic growth factor is TGF-ß1, TFG- ß2, PDGF, IFNγ or IL-1.

5. The use as claimed in claim 2 wherein the therapeutic agent is a non-fibrotic growth factor.

6. The use as claimed in claim 5 wherein the non-fibrotic growth factor is TGFß-3, FGF-1, FGF-2, IL-4 or IL-10.

7. The use as claimed in 2 wherein the therapeutic agent one which is capable of affecting the quantity of active growth factor or a protein associated therewith in a wound site at which the gene product is expressed.

8. The use as claimed in claim 2 wherein the therapeutic agent is IL-4 or IL-10 or a fragment or a partially modified form thereof.

9. The use as claimed in claim 2 wherein the therapeutic agent is a soluble betaglycan or a fragment or an analogue thereof.

10. The use as claimed in claim 2 wherein the therapeutic agent is an inhibitor of Interferon-γ.

11. The use as claimed in claim 2 wherein the therapeutic agent is a stimulator of IFN-γ.

12. The use as claimed in claim 2 wherein the therapeutic agent is an inhibitor of activation of at least one integrin receptor.

13. The use as claimed in claim 2 wherein the therapeutic agent is an inhibitor if at least one convertase enzyme.

14. The use as claimed in claim 2 wherein the therapeutic agent is a stimulator of Activin and/or Inhibin.

15. The use as claimed in claim 2 wherein the therapeutic agent is one which modulates actin assembly and organisation.

16. The use as claimed in claim 2 wherein the therapeutic agent is one which inhibits the activity of Interleukin-6.

17. The use as claimed in claim 2 wherein the therapeutic agent is Latency Associated Peptide or a functional analogue thereof.

18. The use as claimed in claim 2 wherein the therapeutic agent is Insulin Like Growth Factor II or a functional analogue thereof.

19. The use as claimed in claim 2 wherein the therapeutic agent is a compound that influences the sex hormone system.

20. The use as claimed in claim 1 wherein the medicament is for use in the treatment of Ulcerative Colitis or Inflammatory Bowel Disease.

21. The use as claimed in claim 20 wherein the therapeutic agent is selected from Sulphasalazine (and other 5-aminosalicylates), Metronidazole, Corticosteroids, Azathioprine. Cyclosporin A, Methatrexate. Omeprazole. Lansoprazole, and Rabeprazole.

22. The use as claimed in claim 1 wherein the medicament is for use in the treatment of epilepsy.

23. The use as claimed in claim 22 wherein the therapeutic agent is Phenytoin, Phenobarbitone, Sodium Valporate, Topiramite.

24. The use as claimed in claim 1 wherein the medicament is for use in the treatment of reperfusion injury and hypertension.

25. The use as claimed in claim 24 wherein the therapeutic agent is selected from Phosphodiasterase inhibitors, Modulators of immune response/apoptosis, Vasodilators. ACE inhibitors, Fibrinolytic agents, Anti platelets, Anti coagulants, Beta blockers. and Free radical scavangers.

26. The use as claimed in claim 1 wherein the medicament is for the treatment of Cystic Fibrosis.

27. The use as claimed in claim 26 wherein the therapeutic agent is Ibuprofen or Prednisolone.

28. The use as claimed in claim 1 wherein the medicament is for the use in the treatment of Psoriasis.

29. The use as claimed in claim 27 wherein the therapeutic agent is a steroid, Vitamin D analogue, Psoralen, or Antimitotic/immunosuppressant.

30. The use in the manufacture of a medicament for the treatment of Rheumatoid Arthritis of a bioreductive conjugate comprised of a non-cytoxic bioreductive moiety with linked thereto at least one therapeutic agent selected from Sulfasalazine, Mesalazine, Penicillamine. Azathioprine, Chlorambucil, Myochrysine (sodium auro thiomalate), Hydroxychloroquine, Methotrexate, Cyclosporin Myocrisin and Neoral.

31. The use in the manufacture of a medicament for the treatment of Diabetes of a bioreductive conjugate comprising a non-cytoxic bioreductive moiety with linked thereto at least one therapeutic agent selected from Acarbose, Aspirin, Indomethacin, Caprópril and Prostaglandin Synthetase inhibitors.

32. The use in the manufacture of a medicament for the treatment of ischemia of a bioreductive conjugate comprising a bioreductive moiety with linked thereto at least one therapeutic agent selected from Inositol Nicotinate, calcium antagonists, such as Niphedipine and Verapamil; anti platelets, such as Aspirin and Dipyridamole, ACE inhibitors, (Agniotensin Converting Enzyme) e.g. Ramapril and Trandolapril, fibrinolotic agents.

33. A bioreductive conjugate comprising a bioreductive moiety with linked thereto a therapeutic agent selected from Ibuprofen, Naproxen, Fenoprofenb, Benoxaprofen, Sulindac, indomethacin, tolmetin and Diclofenac.

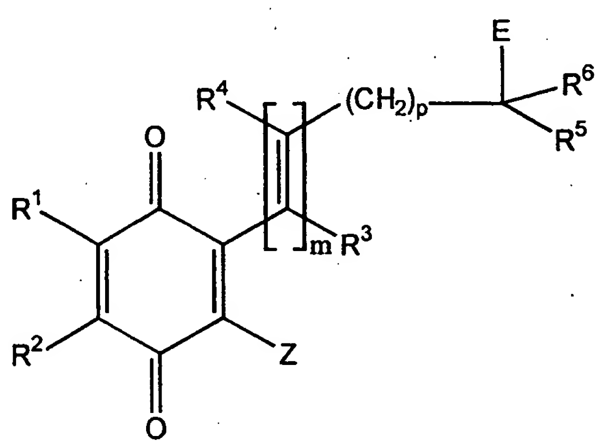
34. A bioreductive conjugate comprising bioreductive moiety with linked thereto a therapeutic agent which is PDE-4 inhibitor.

35. A bioreductive conjugate comprising bioreductive moiety with linked thereto a therapeutic agent which is PDE-5 inhibitor.

36. The use for the manufacture of a medicament for the treatment of a hypoxic condition of a bioreductive conjugate comprising a bioreductive moiety with linked thereto a PDE inhibitor.

37. A bioreductive conjugate comprising a bioreductive moiety with linked thereto a therapeutic agent which is selected from immunosupressives, cell cycle specific drugs, cell cycle non-specific drugs, metalloprotease inhibitors and inhibitors of nitric oxide synthase.

38. The invention as claimed in any one of claims 1 to 37 wherein the conjugate is of the formula (II)



(II)

(wherein

R^1 and R^2 independently represent hydrogen or halogen atoms, or a group R, OR, SR, NHR, NR_2, CO_2R or CONHR;

or, alternatively, R^1 and R^2 together with the intervening ring carbon atoms form a 5-7 membered carbocyclic or heterocyclic ring itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, CO_2R and CONHR;

Z represents an alkyl, alkenyl, aryl or aralkyl group optionally carrying at least one OH, SH, NH_2 or NHR^7 group in which R^7 is an alkyl group;

R^3, R^4, R^5 and R^6 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L;

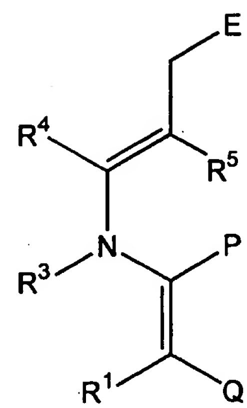
m = 0, 1, 2 or 3; and

p=0 or 2;

with the priviso that when m=1 then p=0)

or a salt thereof.

39. The invention as claimed in any one of claims 1 to 37 wherein the bioreductive conjugate is of the formula (III)



(III)

(wherein

P and Q together with the intervening ring carbon atoms form a quinone or indoloquinone ring, a nitroaromatic, N-oxide or diazoaromatic compound, itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R. OR, SR, NHR, NR21 C02-R and CONHR;

R' represents a hydrogen or halogen atom, or a group R, OR, SR, NHR, NR_2, $C0_2R$ or CONHR;

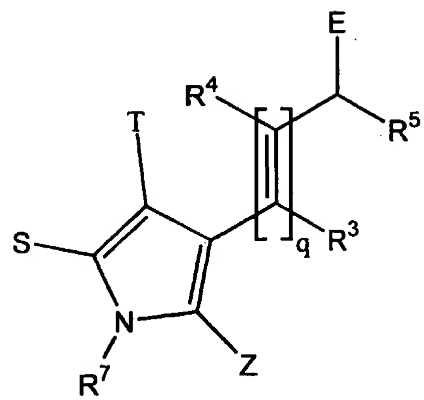
R^3, R^4 and R^5 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group; and

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L)

or a salt thereof.

40. The invention as claimed in any of claims 1 to 37 wherein the bioreduction conjugate is of the formula 1V.



(IV)

(wherein

S and T together with the intervening ring carbon atoms form a quinone or iminoquinone ring, a nitroaromatic or N-oxide compound, itself optionally substituted by one or more halogen atoms, or by one or more groups selected from R, OR, SR, NHR, NR_2, CO_2R and CONHR;

Z represents an alkyl, alkenyl, aryl or aralkyl group optionally carrying at least one OH, SH, NH_2 or NHR^6 group in which R^6 is an alkyl group;

R^7 represents an alkyl group;

R^3, R^4 and R^5 independently represent hydrogen atoms or an alkyl or alkenyl group;

each group R independently represents a hydrogen atom, an alkyl or alkenyl group;

$q = 0, 1, 2$ or 3; and

E represents the residue of a therapeutic agent to be delivered, optionally attached via a linking group L)

Or a salt thereof